Pharmaceutical Research, Vol. 14, No. 11, 1997

Review

Stereoselectivity of Antibodies for the Bioanalysis of Chiral Drugs

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Received April 13, 1997; accepted August 23, 1997

The use of immunoassay techniques represents an alternative approach to the more common chromatographic methods for the determination of the concentrations of chiral drugs. In the development of the former technique, the inherent stereoselectivity of the antibodies used appears to be an important parameter to be studied. The structural features of the drug, including the environment around the asymmetric center, the flexibility of the molecule and the ability of the molecule to undergo racemization, contribute to the molecule's ability to be recognized by stereoselective antibodies. These parameters are intrinsic and can not be influenced by the investigator. On the other hand, other parameters can be modified to favor the raising of highly stereoselective antibodies. These include the synthesis of an appropriate hapten; the selection of an optimal spacer arm between the hapten and the carrier protein used for the immunization procedure; and the choice of appropriate immunization and antibody-screening procedures. The purification of antibodies using affinity chromatography may also facilitate the selection of stereoselective antibodies.

KEY WORDS: monoclonal antibodies; polyclonal antibodies; stereoselectivity; chiral drugs; immunoassay; chiral analysis.

INTRODUCTION

The pharmacodynamic and/or pharmacokinetic properties of the enantiomers of chiral drugs may differ greatly (1). A wide variety of analytical methods have been developed to separate and quantify individual enantiomers. Of these, chromatographic methods are most frequently used whereby enantiomers are converted into diastereomers prior to analysis, or separated on chiral stationary phases (2). In addition, biologically based procedures are also very useful.

Antibodies (Abs) are themselves chiral molecules and the binding between chiral drugs and Abs may involve stereoselective interactions. Not surprisingly, stereoselective Abs have also been raised as tools for the separation and/or quantitation of stereoisomers by enantioselective immunoassays (3).

The production of suitable Abs, in terms of affinity and selectivity towards metabolites, is fundamental to the development of immunoassay procedures for drugs (4,5). In the case of chiral assays, Ab stereoselectivity must be considered as part of the overall selectivity. The raising of Abs against a drug with a molecular weight which is too low to elicit an immune response may be summarized in five steps: (i) firstly, if the molecule lacks suitable functional groups to enable it to bind to an immunogenic carrier, the drug must be modified to introduce appropriate functional groups; (ii) secondly, the hapten must be linked to a carrier protein (the nature and length of this linkage are important considerations); (iii) in the third step, animals—typically rabbits and mice—are immunized with the immunogen (i.e. hapten + carrier protein) to allow the production of polyclonal (PAbs) or monoclonal (MAbs) antibodies; (iv) in the fourth step, the screening of specific Abs is performed and (v), the resulting Abs are finally characterized in terms of their affinity and selectivity in the remaining step.

The stereoselectivity of Abs reflects their ability to differentiate between the different configurations. This property appears to be linked to the accessibility of a molecule's asymmetric center(s), and its contribution in forming the antigenic determinant. Regardless of the field of application, the selection of stereoselective Abs may be influenced by several parameters during each of the steps leading to antibody production. The main factors include: the primary structure of the drug; the use of the racemate or a single enantiomer for immunization; the enantiomeric purity of the drug; its physico-chemical properties, (including the chemical reactivity of each configuration, and the length of the linker arm between the hapten and the carrier protein); the structural environment of the chiral center in the immunogen formed; the nature of the Abs (monoclonal or polyclonal) and the choice of the labelled antigen used for the antibody screening procedure. Of the factors governing antibody stereoselectivity, some depend on the drug itself and others may be controlled by the investigator.

The aim of this review is to discuss these factors, in order to define a general strategy which may lead to the production of stereoselective Abs.

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0724-8741/97/1100-1516$12.50/0

INTRINSIC FACTORS GOVERNING ANTIBODY STEREOSELECTIVITY

Racemization or Chiral Inversion

Failure to raise stereoselective Abs against a single enantiomer can be due to the fact that the molecule undergoes spontaneous racemization or chiral inversion.

Oxazepam is a good example of this, undergoing racemization in the pH range of 6 to 8 at 20°C (6). Chiral inversion can also be induced during the preparation of the conjugates, as was discovered for atropine (3).

Location of Asymmetric Center

When the asymmetric center is part of a ring, the reduced flexibility of the structure may in some cases favor the stereoselective recognition by Abs.

As an example, antisera elicited against both pentobarbital enantiomers showed cross-reactivities of up to 1.4% towards opposite configurations (7), compared to the 0.0005–0.005% cross-reactivity obtained with Abs raised against enantiomers of hexobarbital, a structural analogue of pentobarbital in which the asymmetric center is part of a ring (8).

The flexibility of functional groups in the vicinity of asymmetric centers may therefore influence the likelihood of obtaining stereoselective Abs. To test this hypothesis, it appears logical to produce Abs directed towards a molecule having at least one chiral center in a flexible moiety (a lateral chain for example) and compare these with those produced towards a second chiral center on a more rigid moiety, and to study the cross-reactivities for each configuration.

Unfortunately, little data is available related to Abs directed towards such molecules and it is difficult to determine to what extent differences in stereoselectivity may be attributed to the location of the asymmetric center, or to the proximity of chiral centers to the carrier proteins of immunogens used to obtain Abs. The limited information available will be discussed in the following sections.

Influence of Functional Groups Bound to the Asymmetric Center

The nature, and/or the spatial orientation of functional groups bound to the asymmetric center may contribute to the overall immunogenicity of the drug and to the stereoselectivity of the elicited Abs.

Some clear-cut examples illustrate the relationships between immunogenicity and configuration. For example, preferential recognition of one configuration at the expense of the opposite was found for anti-cyclazocine Abs (9). Twelve antisera were obtained from four *d,l*-cyclazocine/BSA derivatives used for the immunization procedure: ten out of the twelve were more selective towards the *l*-configuration.

SK&F 94836 provides another example, with both enantiomerical configurations used for the immunization procedure (10). The Abs raised in response to the *R*-(−) enantiomer did not cross-react with *S*-(+)-SK&F 94836 to any significant extent, while the Abs raised in response to the *S*-(+) form cross-reacted to the extent of 10% with *R*-(−)-SK&F 94836.

This preferential recognition of one configuration can be explained by the hydrophobicity and the spatial arrangement of the groups carried by the asymmetric center, as shown with oxaprotiline (11). Cross-reactivity experiments carried out with oxaprotiline analogues modified on the chiral center showed that the tetracyclic structure of oxaprotiline, together with the aliphatic side-chain containing the asymmetric carbon atom, are complementary to a continuous shallow hydrophobic pocket on the Ab combining site. The authors suggested that the stereoselective recognition of oxaprotiline could be directly dependent upon the spatial positioning of the hydroxyl group on the asymmetric carbon (Fig. 1). Analysis of the immune response showed that only the (−)-isomer was able to induce high affinity Abs with a certain stereoselectivity, while the (+)-isomer, even when it was used for the preparation of the immunogen, only induced low affinity Abs.

Hydrophobicity of the Epitopic Region

The hydrophobicity of the antigenic combining site also appears to be a major factor in governing antibody stereoselectivity. For soman, a racemic mixture of isomers with two asymmetric centers (one on carbon, C(±), and one on phosphorus, P(±), Figure 2), certain findings indicate that primary antibody selectivity is directed towards the chiral phosphorus atom, since the highest affinity was measured for the P(−)-isomers (12). In spite of the presence of a second asymmetric center, located on the pinacolyl group, the C(+) configuration demonstrated the best interaction in combination with the P(−) configuration, while the C(−) configuration bound best in conjunction with the P(+) configuration (selectivity varied as C(+)P(−) > C(−)P(−) > C(−)P(+) > C(+)P(+)). Overall, the configuration of the chiral phosphorus atom plays a dominant role in the binding of soman derivatives to Abs, while the chiral contribution of the pinacolyl group is eclipsed by its hydrophobicity (despite it being further away than the phosphorus atom from the linkage site between the hapten and the carrier used for the immunization).

The location and the polarity of functional groups is clearly important for the stereorecognition of Abs. In order to predict chiral ligand-antibody interactions, molecular modeling studies could be carried out, as was suggested for ligand-receptor interactions (13).

Affinity and Stereoselectivity

The presence of an asymmetric center in a drug implies the potential existence of different configurations. For configurational recognition, at least three simultaneous interactions between antigens and Abs must take place. In a general way, low affinity seems to rule out high stereoselectivity, resulting from the lack of multiple interactions between antigens and Abs. This raises the question of a possible relationship between affinity and stereoselectivity.

As an example, the modest stereoselectivity of Abs towards soman may be related to the results which indicate that only two loci on the molecule (the phosphonyl oxygen and the *t*-butyl group) provide sources of interactions with the antibody (12,14). Results in accord with this hypothesis were found with Abs elicited for nicotine and cotinine (15). In this particular study, the anti-*S*-(−)-nicotine MAbs cross-reacted with *R*-(+)-nicotine for 3.9%, while the anti-*S*-(−)-cotinine Abs exhibited less affinity for the nicotine structure and showed similar cross-

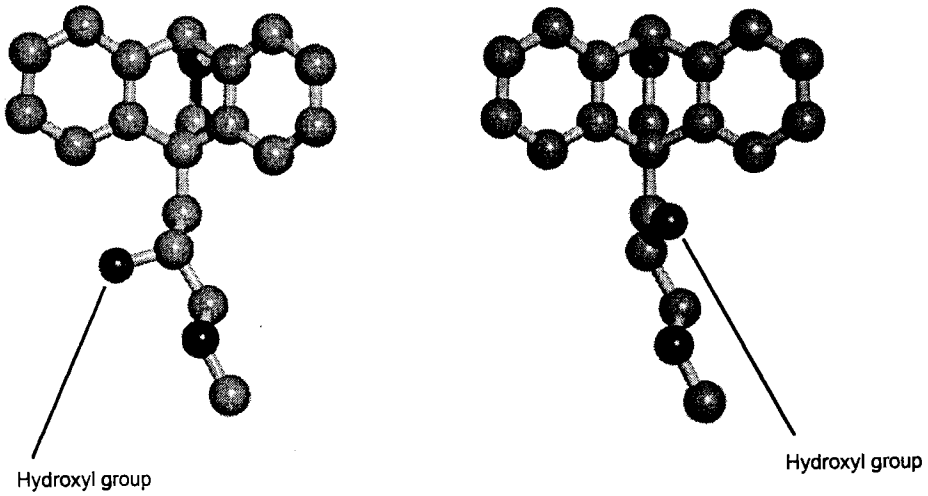


Fig. 1. Oxaprotiline isomers (1). Both conformers have been optimized using Hyper Chem™ (Hyper Chem version 5.01, Hypercube Inc., USA), and the images transformed using POV-Ray™, version 3.00e. In the (−)-isomer (in the left of the figure), the hydroxyl group on the asymmetric carbon atom is pointing away from the hydrophobic surface of the molecule recognized by the MAb (from ref. 11). Hydrogens have been removed for added clarity.

reactivities for both nicotine configurations. A similar relationship between affinity and stereoselectivity has been observed for alprenolol: out of the four types of MAbs produced, the one with the lowest binding affinity did not exhibit ligand stereoselectivity (16).

Environment and Topological Profile Around the Asymmetric Center

The environment around the asymmetric center may also influence the stereoselectivity of antibodies. Some chiral molecules may exhibit entirely different topological profiles in their different configurations, and others may not. With regards to stereoselectivity, Jacques (17) reported that the similarity between the smells of small volatile chiral molecules could be attributed to the fact that these molecules are quasi-spherical, hence not very asymmetric. Therefore, owing to the very high spatial likeliness of both enantiomers, olfactive receptors are not capable of telling them apart. In the light of the analogy between receptors and Abs in terms of interactions with ligands (specific, saturable and reversible interactions), the stereoselectivity of Abs is also dependent upon the spatial arrangements of groups around the asymmetric center and/or the topology of chiral molecules.

Higher Chemical Reactivity of One Configuration

The hapten/carrier molar ratio has been tentatively reported to influence the titre and selectivity of Abs. An optimal hapten/carrier ratio can be found for an enantiomer when its linkage to the carrier is easier than its antipode: this was proposed for atropine (18). The same was also true when stereoselective PAbs were produced for methadone. In this case, the ratio of methadone to thyroglobulin was higher for the α-derivatives in comparison with the β-derivatives (19).

CONTROLLABLE FACTORS GOVERNING ANTIBODY STEREOSELECTIVITY

Influence of the Chemical Modification Transforming the Drug into the Hapten

Some compounds do not possess the appropriate functional groups needed to couple them to a carrier. As a consequence,

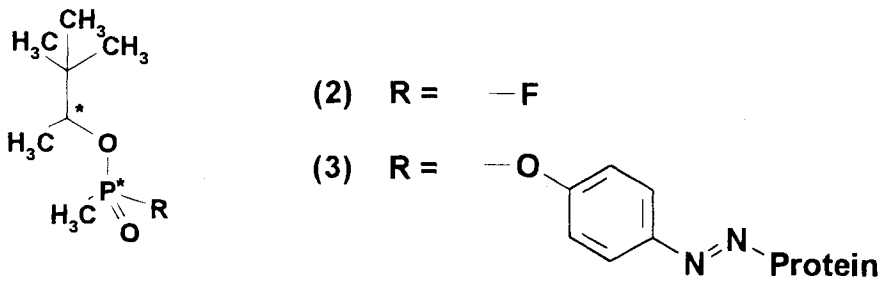


Fig. 2. Structural formulae of soman (2) and its conjugate (3) (from ref. 12). * denotes asymmetric centers.

derivatives must be synthesized to incorporate such groups. This step is known as hapten synthesis.

Although most drugs do not undergo spontaneous racemization, it can occur following the modification step for the production of the hapten. This has been evoked for Abs raised against nicotine using 6-(p-aminobenzamido)nicotine as the hapten (3). The investigator must therefore take this possibility into account and beware of chiral inversion during the hapten synthesis.

The investigator must also be aware that the affinity and overall selectivity of the resulting Abs may be influenced by modifying chemical groups of the parent drug. Evidence of this can be found in a study designed to produce Abs for the development of a stereoselective radioimmunoassay of *d*- and *l*-methadone, using α-*l*-methadol-hemisuccinyl-thyroglobulin as the immunogen (20). The same Abs were used to develop a stereoselective radioimmunoassay of α-*l*-acetylmethadol. These Abs showed better affinity for α-*l*-acetylmethadol, which is more closely related to the hapten, than methadone (21). It appears that affinity, hence stereoselectivity, is increased when the hapten structure closely resembles the original molecule, or *vice versa* when the drug antigen closely resembles the protein-drug conjugate.

Influence of the Hapten/Carrier Protein Coupling Site

Abs resulting from conjugates are generally more specific for the parts of the molecule which are not involved in binding with the protein (22). The relationship between the antiserum selectivity and the structure of the immunizing conjugate is well documented in the narcotic alkaloid series. The coupling of morphine at the 2- or 3- or 6-position elicited antisera of wide selectivity which could be used for forensic science studies (23). In contrast, coupling of morphine to a protein through the piperidine ring nitrogen resulted in highly specific antisera which only reacted with morphine and which were appropriate for pharmacokinetic studies (24).

For chiral molecules, several studies have shown that stereorecognition is improved when the asymmetric center is further away from the coupling site. This may be illustrated in the case of enprostil (Fig. 3), a prostaglandin analogue (25). No notable differences were found in the binding behavior towards the antiserum elicited against enprostil for the stereoisomers that differed only at the allenic stereocenter (the nearest stereocenter to the coupling site between the hapten and the keyhole lympet hemocyanine used as the carrier protein). In contrast, the Abs showed a clear preference for the *SSSS* configuration at the four remaining non-allenic stereocenters. These results are reinforced by those obtained from an antiserum raised against the 86505-007 isomer, presenting the *R*-(*RRRR*) configuration. Once more, although only one single configuration was used for the immunization procedure, none of the Abs could make the distinction between the *R*-(*RRRR*) and the *S*-(*RRRR*) configurations.

Other drugs with two or more asymmetric centers, such as *d*-pseudoephedrine or α-*l*-acetylmethadol, also provide good examples of this phenomenon (19,26).

Influence of the Length and the Nature of the Spacer Arm

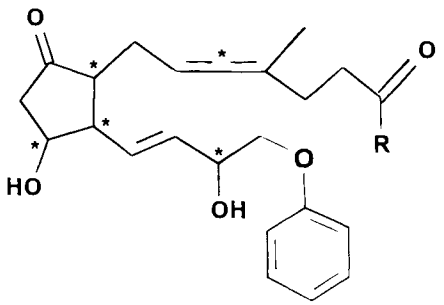
1. The *length* of the spacer arm needs to be discussed, with consideration given to the chiral centre location. If the chemical group involved in the link between the hapten and the carrier is bound to the asymmetric center, it is advisable to increase the distance between the asymmetric center and the carrier protein so as to increase the probability of raising Abs with chiral recognition properties.

The hemisuccinate derivative of oxaprotiline, produced through coupling with the hydroxyl group on the asymmetric carbon spacer arm, was used to produce anti (−)-oxaprotiline MAbs (11). Only four out of the twelve MAbs produced were selective for the (−)-configuration, while five out of the twelve recognized both (−)- and (+)-oxaprotiline (one was even selective for the opposite configuration). These results were found despite better recognition of the (−)-configuration with PAbs raised against the racemate. This point underlines the importance of choosing the right distance between the asymmetric center and the coupling site to tentatively improve stereorecognition.

Conversely, when the chiral center is already distant from a possible coupling site, it is preferable to reduce the length of the arm between hapten and protein carrier to preserve affinity. Some examples demonstrate that a long spacer is not compatible with a high affinity for the free drug (26,27).

2. The *chemical nature* of the spacer arm is also likely to be chosen on the basis of a compromise between stereorecognition and affinity.

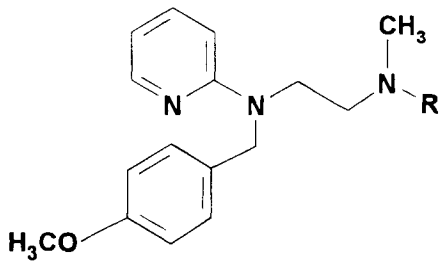
L. Chatenoud *et al.* showed that anti-SK&F 94461 MAbs (a specific H_1 histamine receptor ligand) recognized SK&F 94461 and mepyramine with near equal affinities (28). Mepyramine differs from SK&F 94461 in that it lacks the butylamine group which lengthens the spacer arm. As the hapten was a hemisuccinate derivative of SK&F 94461 (Fig. 4), it is likely that methylene groups, which are not immunogenic, do not belong to the epitopes recognized by the Abs. Methylene spacer



(4) R = OCH3

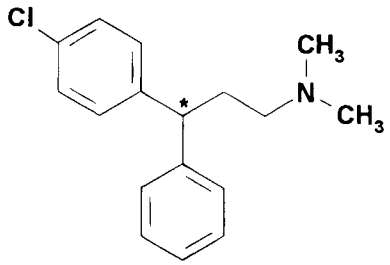
(5) R = NH-Protein

Fig. 3. Enprostil (**4**) and immunogen (**5**) used in ref. 25. * denotes asymmetric centers.



(6) R = $-(CH_2)_5-NH_2$

(7) R = $-CH_3$



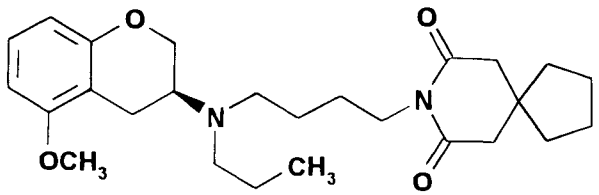
(8)

Fig. 4. Chemical structures of SK&F 94461 (**6**) and analogues: mepyramine (**7**) and chlorpheniramine (**8**); * denotes the asymmetric center.

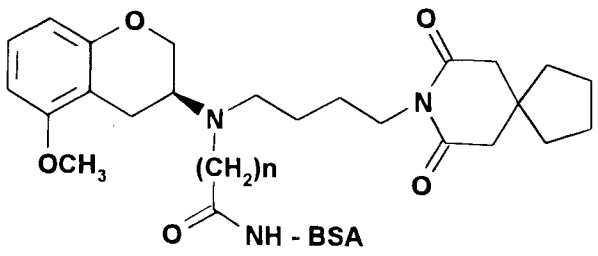
groups would therefore appear to be useful to distance asymmetric centers from the carrier molecules.

Through the same reasoning, highly immunogenic groups, sometimes used for coupling the hapten to the carrier, can be favourably recognized by the elicited Abs. This increases the risk of not obtaining at least three interactions between the original ligands to be assayed and the Abs, precluding good stereoselectivity.

As a general conclusion, the stereoselectivity of elicited Abs tends to be better when the structure of the hapten is similar to the structure of the molecule being assayed. Whenever a spacer arm is required, it is preferable to use a lipophilic non-immunogenic structure which is as short as possible. This reasoning was applied to the production of stereoselective PAbs against S 20499, a potent 5 HT_{1a} agonist currently under development at the Servier group. Two haptens were prepared: one a derivative resembling the original structure of S 20499, with the effective addition of a carboxylic acid group, and a second with the effective addition of a butanoic acid moiety, believed to favor stereorecognition (Fig. 5). Both approaches gave high levels of stereoselectivity, with cross-reactivities towards the optical antipode of S 20499 of less than 0.1% for four out of the six antisera produced (29).



(9)



Immunogens : **(10)** n = 3

(11) n = 6

Fig. 5. Structural formulae of S 20499 (**9**) and its conjugates (**10** and **11**), described in ref. 29.

Immunization and Screening Procedures

The selection of the hapten configuration used for the synthesis of the immunogen and the antigen used for the screening of elicited Abs are important points to consider to improve the stereoselectivity.

Four possible combinations of immunogens and screening antigens may be studied:

i) a racemic form may be used both for the immunogen and the screening antigen,

ii) a racemic form may be used for the immunogen preparation, and an enantiomeric configuration for the screening antigen,

iii) an enantiomeric configuration may be used for the immunogen preparation and a racemic form for the screening antigen,

iv) an enantiomeric form may be used both for the immunogen preparation and the screening antigen.

Each of these combinations lead to heterogeneous results. Nevertheless, a development strategy should be considered which takes into account the ultimate purpose for which the Abs are intended: assaying racemates or individual enantiomers.

Antibodies for Racemate Assays

The use of racemates for both immunization and screening procedures has been developed and seems to be preferential for developing immunoassays of racemic drugs (30,31). However it is wrong to assume that Abs produced in this way are automatically non-stereoselective. This immunization scheme results in a mixture of antibody families produced by several clones, expressing a selectivity pattern which is heterogeneous. Abs themselves are chiral and each MAb is likely to bind the enantiomers of a mixture to a different extent, as found, for instance, for Abs against atropine (16) or propranolol (32).

MAbs developed against alprenolol clearly illustrate the different responses which can be obtained from various clones (33). In this study, both the immunization and characterization of Abs were performed with the racemate of alprenolol. Out of the four MAbs obtained, two recognized the *l*- and *d*-configurations of propranolol (a structural analogue of alprenolol) to an equal extent, one recognized the *l*-form twice as readily as its opposite counterpart in terms of cross-reactivity, while for the last antibody, the observed affinity for *d*-propranolol was lowered by a factor of twenty.

There are other ways of establishing reliable racemate assays. Since an antiserum is a polyclonal mixture of several Abs descended from sensitized clones, some authors have tried to develop mathematical models to describe these systems. Rominger and Albert (34) proposed a system of equations based on coupled mass equilibria, corresponding to m Abs with one binding site, or with several mutually independent binding sites of equal intrinsic affinity, and n monovalent antigens. This model, which describes the behavior of a system based on a mixture of stereoselective Abs (for $m = 2$ and $n = 4$) and a racemic tracer, has been applied to the radioimmunological determination of racemic fenoterol (35).

To avoid the production of Abs which recognize one configuration to a greater extent than another, Cook et al. suggested using a hapten which resembles the drug being assayed, in which the asymmetric center (or the chirality) is removed (36). This approach gave good results during the development of a racemic immunoassay for WR 171,669, an antimalarial drug. Once again, due to the chiral nature of Abs, it is not easy to obtain Abs which lack stereoselectivity. Other studies, which made use of achiral conjugates for immunization, illustrate the possibility of producing effective stereoselective PAbs and MAbs (28,37).

Antibodies for Enantiomer Assays

Using a Racemate for the Preparation of the Immunogen and a Single Configuration for the Screening Antigen It is possible to obtain Abs with appropriate stereoselectivity by combining a racemate form in the immunogen and an enantiomeric antigen for antibody screening. Although this does not seem to be the best way of obtaining stereoselective Abs, some groups have resorted to this system. A study based on nicotine illustrated the possibility of exploiting the inherent stereoselectivity in the screening assay (15). Stereoselective MAbs were prepared against nicotine in the racemic form. To optimize selection of selective Abs, hybridomas were screened by immunoprecipitation using tritium-labelled *S*-(−)-nicotine, corresponding to the naturally occurring isomer. For the nine selected and cloned hybridomas, a 5 to 45-fold increase of *R*-(+)-nicotine was required for 50% inhibition in comparison with the naturally occurring isomer, underlining the preferential recognition of each antibody for *S*-(−)-nicotine.

Using this method, there is nonetheless a high probability of obtaining antibody-secreting clones which are sensitized against both configurations. In the case of MAb production, it is possible (in theory) to isolate some highly selective clones of each configuration by immunizing with a racemate. Using an enantiomerically pure labelled antigen for screening should allow the selection of these stereoselective clones.

Using One Single Configuration for the Preparation of the Immunogen and a Racemate Form for the Screening Antigen The use of an enantiomer for the immunization procedure and a racemic form for the antigen used to select Abs is another way of producing stereoselective Abs. Although this method is rarely used, it could be of value if a radiolabelled enantiomer is not available. Since Abs are raised towards one configuration, they might be considered unlikely to cross-react with the opposite enantiomer. Hence, the elicited Abs are more likely to preferentially recognize the antigen configuration against which they are produced. Cross-reactivity for the opposite configuration remains variable and depends, in most probability, on the overall structure of the molecule: for example, very high stereoselectivity has been obtained using this method for pseudoephedrine or ephedrine (26,38), with cross-reactivities of the optical antipode of less than 0.05%, and medium stereoselectivity for (−)-nicotine (3.9%) (39) or (+)-propranolol (7.2%) (40).

Using One Single Configuration for the Preparation of the Immunogen and One Single Configuration for the Screening Antigen Highly stereoselective PAbs or MAbs are more often obtained using both enantiomers for immunogens and also for screening antigens. This technique has been applied for a whole variety of compounds such as phospholipids (41), prostaglandins (42), insecticides (43) and pharmaceuticals including pentobarbital (7), α-(−)-acetylmethadol (21), warfarin (44), loxoprofen (45), propranolol (46), aprikalim (47). The highest degree of enantioselectivity is likely to be achieved when both the immunogen and the labelled ligand are enantiomerically equivalent and optically pure (3). Enantiomerically pure immunogens favor the formation of enantioselective antibodies and enantiomerically pure labelled ligands improve the enantioselectivity of the assay even further.

Polyclonal Versus Monoclonal Antibodies

From the authors' own findings, there appears to be no definite advantage in using MAbs or PAbs as far as stereoselectivity is concerned. Nonetheless, in a sampling of 42 papers in which cross-reactivity results of individual stereoisomers were detailed, 75% dealt with PAbs and 25% with MAbs. Antisera

have the advantage of producing Abs with higher affinities. Moreover, when a racemate is to be analysed, the presence of a mixture of antibody families allows both configurations to be recognized more easily. Of the 42 papers, only one single MAb was produced to develop a racemate assay. This figure may be compared with 9 out of the 31 for PAbs elicited for the same purpose, illustrating the advantage of developing racemate assays using PAbs. MAbs belong to a unique and homogeneous class of Abs: their advantages and limitations to hapten immunoassays have been reported (4). It seems more logical to use them for stereospecific immunoassays, to avoid minor cross-reaction of the type found for PAbs, where the production of antibody families exhibiting low affinities for the opposite enantiomer is common. Moreover, the possibility of developing a large number of MAbs from a single fusion can help in the selection of one with the required stereoselectivity.

Purification of Antibodies

After producing PAbs, a procedure may be employed to exclude reagents (i.e some of the Abs produced) which increase the risks of giving high cross-reactivities. As cross-reactivity is related to heterogeneous populations of Abs, each with different stereoselectivities, one may exploit chromatography through the use of affinity columns to isolate Abs which are more stereoselective. This has been generally demonstrated (48).

CONCLUSIONS

No single, universal method is available to increase the stereoselectivity of Abs: only general approaches can be given. Since drug structures and the unpredictable responses of animals towards immunization procedure are factors which cannot be fully controlled, investigators must optimize the remaining controllable parameters to increase antibody stereoselectivity. The key to success appears to be in choosing optimal hapten structures, for immunogen synthesis and assay-screening procedures which are adapted for the selection of desired Abs. Appropriate decisions taken at each step may help to increase the probability of achieving the desired stereoselectivity sought in accordance with the objectives of the immunoassay. Owing to their sensitivity, simplicity and ability to batch-process large numbers of clinical samples, stereoselective immunoassay techniques still remain interesting analytical tools (47,49). They also enjoy the status of being relatively inexpensive, and as sensitive as the more sensitive instrumental methods such as gas or liquid chromatography coupled to mass-spectrometry detection (50). While the main limitation of drug immunoassay methods stems from the lack of antibody selectivity (51), excellent correlation can be found between immunological and instrumental methods (50).

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